JCM
Journals.ASM.org

Validation of Rapid Point-of-Care (POC) Tests for Detection of Hepatitis B Surface Antigen in Field and Laboratory Settings in the Gambia, Western Africa

Harr Freeya Njai,[a] Yusuke Shimakawa,[a,b] Bakary Sanneh,[c] Lynne Ferguson,[a] Gibril Ndow,[a] Maimuna Mendy,[d] Amina Sow,[e] Gora Lo,[e] Coumba Toure-Kane,[e] Junko Tanaka,[b] Makie Taal,[c] Umberto D'alessandro,[a] Ramou Njie,[a,d] Mark Thursz,[f] Maud Lemoine[a,f]

Medical Research Council Unit, the Gambia, Banjul, the Gambia[a]; Department of Epidemiology/Infectious Disease Control and Prevention, Hiroshima University, Hiroshima, Japan[b]; National Public Health Laboratory, Banjul, the Gambia[c]; Laboratory Services and Biobank Group, International Agency for Research on Cancer, Lyon, France[d]; Laboratoire de Bacteriologie Virologie, Université Cheikh Anta Diop, CHU Le Dantec, Dakar, Senegal[e]; Department of Hepatology, Division of Medicine, Imperial College, London, United Kingdom[f]

Hepatitis B virus (HBV) infection is a leading cause of death in sub-Saharan Africa (SSA). Point-of-care tests for hepatitis B surface antigen (HBsAg) could be an ideal tool for a large-scale HBV screening/treatment program in SSA. Using data from the PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa) program, we conducted a cross-sectional study to assess the diagnostic accuracy of three point-of-care tests (Determine, Vikia, and Espline) for the detection of HBsAg in the field or a laboratory setting in the Gambia. In the field, we used finger-prick whole blood for the Determine and Vikia tests and dried blood spots for the reference standard test (AxSYM HBsAg enzyme-linked immunosorbent assay [ELISA]). In the laboratory we used serum for the Determine, Espline, and reference test (Architect chemiluminescent microparticle immunoassay). Of 773 participants recruited at the community and 227 known chronic HBV carriers (1,000 subjects in total), 293 were positive for HBsAg. The sensitivity and specificity of the Determine test were 88.5% and 100% in the field and 95.3% and 93.3% in the laboratory setting, respectively. The sensitivity and specificity were 90.0% and 99.8% for the Vikia test (in the field) and 93.9% and 94.7% for the Espline test (in the laboratory). There was no evidence that one kit was better than another. Most of the patients with false-negative results (18/19) were classified as inactive chronic carriers. In summary, the three point-of-care tests had acceptable ranges of diagnostic accuracy. These tests may represent accurate, rapid, and inexpensive alternatives to serology testing for the screening of HBV infection at field level in SSA.

Sub-Saharan Africa (SSA) is an area of high endemicity for hepatitis B virus (HBV) infection, particularly in West Africa, where the prevalence of hepatitis B surface antigen (HBsAg) may exceed 8% (1). HBV is the main cause of hepatocellular carcinoma (HCC), one of the most frequent cancers in SSA and the leading cause of cancer deaths in West African males (2, 3). Despite a highly effective vaccine, people who had established chronic HBV infection before the immunization program are left with a high risk of developing HCC (4). Therefore, immunization alone is not sufficient to control HBV infection (5). To reduce the burden of HBV-related liver diseases and HCC in SSA, identifying infected subjects is essential (5). Yet screening for HBV infection and access to care and treatment are sorely lacking in SSA, where the vast majority of infected individuals are unaware of their serological status.

HBsAg is a key marker for the diagnosis of HBV infection. An enzyme-linked immunosorbent assay (ELISA) is the gold standard to detect HBsAg but requires a high-quality laboratory, expensive equipment, cold storage, well-trained technicians, and a sustained supply of electricity (6). In contrast, point-of-care (POC) tests are easier to use and inexpensive compared with ELISA. In addition, some POC tests accommodate not only serum or plasma but also whole blood collected by finger stick, which can avoid a phlebotomy. Recently, two systematic reviews of POC tests for HBsAg detection confirmed their excellent diagnostic accuracy (6, 7). Taken together, HBsAg POC tests seem suitable for community-based and large-scale screening. However, most of the studies which validated their performance were done at the facility level and among specific populations, i.e., blood donors (8–10), hospital patients (11–13), or HIV-infected people (14–18). Although one study in France investigated the accuracy of HBsAg POC tests in the general population, the tests were performed in health centers using whole blood collected by venipuncture (19).

The ongoing PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa [www.prolifica.eu and https://clinicaltrials.gov/ct2/show/record/NCT02129829; accessed 10 January 2015]) program aims to demonstrate that HBsAg screening at the community level and provision of antiviral treatment decrease the incidence of HCC in West Africa. Within this project, the diagnostic accuracy of three POC tests (Determine, Vikia, and Espline) was estimated, both in the field (using whole blood through finger prick) and laboratory (using serum), and the characteristics of individuals with false-negative results were identified.

Received 20 October 2014 **Returned for modification** 28 November 2014
Accepted 20 January 2015

Accepted manuscript posted online 28 January 2015

Citation Njai HF, Shimakawa Y, Sanneh B, Ferguson L, Ndow G, Mendy M, Sow A, Lo G, Toure-Kane C, Tanaka J, Taal M, D'alessandro U, Njie R, Thursz M, Lemoine M. 2015. Validation of rapid point-of-care (POC) tests for detection of hepatitis B surface antigen in field and laboratory settings in the Gambia, western Africa. J Clin Microbiol 53:1156–1163. doi:10.1128/JCM.02980-14.

Editor: A. M. Caliendo

Address correspondence to Maud Lemoine, m.lemoine@imperial.ac.uk.

H.F.N. and Y.S. contributed equally to this article.

doi:10.1128/JCM.02980-14

MATERIALS AND METHODS

Study participants. We collected the data from three studies within the PROLIFICA program in the Gambia. These studies were approved by the Gambia Government/Medical Research Council (MRC) Joint Ethics Committee (L2013.14) and were conducted in agreement with the principles of the Declaration of Helsinki.

Study 1. From January to December 2012, the PROLIFICA program screened for HBsAg 3,068 adults living in 27 randomly selected local communities using the Determine POC test (Alere, USA). After a community sensitization meeting, a screening site was set up at the center of each community; this could be at a school, mosque, or bantaba (local community gathering space in the open air). Following written consent, Determine was performed using whole blood from finger prick at the screening site. Dried blood spots (DBS) were also collected from all the study participants. The result of the POC test was available within 15 min, and posttest counseling was provided on site. Of the 3,068 DBS collected at the community screening, 10% were randomly selected to be tested with the AxSYM HBsAg ELISA (Abbott, USA) as a reference standard for HBsAg detection and included in the current validation study. The selection was made irrespective of the result of the Determine test. All participants positive by the Determine test and people with false-negative results (nonreactive Determine test but positive ELISA) were invited for a standardized liver assessment at the liver clinic at the MRC. This included physical examination, abdominal ultrasound, transient elastography (FibroScan; Echosens, France), measurement liver function (Vitros 350 Analyzer; Ortho, USA), hepatitis B e antigen (HBeAg) ELISA (ETI-EBK Plus; DiaSorin, Italy), anti-hepatitis C virus (HCV) (AxSYM, anti-HCV; Abbott, USA) and anti-hepatitis D virus (HDV) (ETI-AB-Deltak-2; DiaSorin, Italy) assays, anti-HIV enzyme immunoassay (EIA) (Genscreen Ultra; Bio-Rad, USA), and quantification of HBV DNA and HBsAg using serum.

Study 2. Between August and November 2013, 489 adults living in six randomly selected communities (different from those of study 1) in the Gambia were screened in the field for HBsAg using two POC tests at the same time: Determine and Vikia (bioMérieux, France). DBS were also collected during the screening, and all the DBS were tested by AxSYM HBsAg ELISA. All participants positive for at least one of HBsAg tests (Determine, Vikia, or AxSYM HBsAg ELISA) were invited for liver assessment as described above.

Study 3. Following historical community sero-surveys for HBV infection conducted in rural villages (different from those in studies 1 and 2) in the Gambia in the 1980s (20, 21), there was a cohort of 405 chronic HBV carriers who had been regularly followed for HBV serology. Between May 2012 and April 2014, 301 known chronic HBV carriers agreed to take part in the liver assessment of the PROLIFICA program. After the venipuncture at the liver clinic, serum samples were quantified for HBsAg using a chemiluminescent microparticle immunoassay (CMIA) (Architect; Abbott, USA). The first 227 consecutive serum samples from the same visit were also tested by POC tests in the laboratory, either with Determine or Espline (Fujirebio, Japan), and were included in this validation study.

Rapid POC tests. The Determine, Vikia, and Espline POC tests were evaluated for their diagnostic accuracy to detect HBsAg using finger-stick whole-blood samples (Determine in studies 1 and 2 and Vikia in study 2) or serum (Determine and Espline in study 3). When a test was invalid, it was repeated until a valid result was obtained. The frequency of the invalid result was recorded in study 2.

Fieldworkers had 2 days of training for the use and storage of POC tests according to the manufacturers' instructions. In study 2, results of the POC tests were read and recorded independently by one laboratory staff member and one fieldworker; only results on which the two readers agreed were included. In contrast, the results were read by one fieldworker in study 1 and by one laboratory technician in study 3.

DBS. Five drops of finger-stick whole blood were absorbed onto a filter paper (Whatman 903) which was dried for 2 h in the field and then overnight at room temperature at the MRC laboratory and eventually stored in plastic bags with desiccant at room temperature. A 6-mm disc was punched from the dried blood spots (DBS), and elutes were obtained according to the manufacturer's instructions and stored at −20°C.

Reference standard tests for HBsAg detection. For study 1 and 2, the AxSYM HBsAg ELISA was performed as a reference standard on whole-blood samples eluted from DBS. Positive results were confirmed by neutralization assay as per the manufacturer's instructions. For study 3, sera were quantified for HBsAg using Architect HBsAg CMIAs. All the samples with ≥0.05 IU/ml were classified as positive for HBsAg, according to the manufacturer's protocol. Laboratory technicians were blinded to the results of the POC tests.

HBsAg quantification. HBsAg quantification was performed using a CMIA (Architect, Abbott, USA). A 1:500 dilution of samples was performed when the initial value exceeded 250 IU/ml. All samples were run in duplicate.

HBV DNA quantification. HBV DNA was extracted from serum with a Qiagen kit (Qiagen, Hilden, Germany), and a quantitative in-house PCR assay was run using an ABI 5700 sequence detection system. Quality control was ensured by a French laboratory (INSERM, Lyon, France).

Transient elastography. Liver stiffness measurement (LSM) was performed using FibroScan according to previously described technical and examination procedures (22) under fasting conditions (23). For assessing the severity of the liver fibrosis, we used previously published cutoffs (24).

Statistical analysis. Results of POC tests were compared to the result of ELISAs (studies 1 and 2) or CMIAs (study 3). Sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were estimated. The areas under the receiver operating characteristic curves (AUROC) were obtained and compared between POC tests using a test of equality of AUROC. Interrater reliability was determined using the kappa statistic in study 2. Clinical and virological characteristics of study participants with false-negative results (i.e., with at least one negative POC but a positive result with the reference standard) were compared with those of participants with true-positive results (i.e., positive results from both POC and the reference standard). Age, quantified HBsAg levels, HBV DNA levels, alanine aminotransferase (ALT) levels, and LSM results are presented as medians (range), and comparison was made using a Wilcoxon rank sum est. The proportion of males and participants with positive HBeAg was compared between true positives and false negatives using Fisher's exact test. For these comparisons, participants in studies 1 and 2 were combined. All the analyses were performed using STATA, version 11.0 (Stata Corporation, College Station, TX). This study was reported in accordance with the standards for reporting of diagnostic accuracy (STARD) checklists (25).

RESULTS

Study participants. A total of 1,000 participants from the three studies were included in the analysis (296 participants in study 1, 477 in study 2, and 227 in study 3). In study 1, a total of 3,068 people were screened using Determine, and from these 10% of participants (307) were randomly selected. Eleven DBS were lost, and thus 296 DBS were tested by HBsAg ELISA and included in the analysis. The median age of the subjects was 40 years (range, 30 to 105 years), and 113 subjects (38.2%) were male. In study 2, both the Determine and Vikia tests were performed in 489 people. As 12 DBS were lost, 477 DBS were tested by HBsAg ELISA; the median age of the subjects was 43 years (range, 30 to 103 years), and 176 subjects (36.9%) were male. In study 3, both POC tests (either Determine, Espline, or both) and HBsAg serology were performed in 227 subjects. Their median age was 37 years (range, 14 to 79), and 97 (42.7%) were male.

Invalid POC results (study 2). Successful results were obtained for 100% (477/477) and 99.8% (476/477) on the first attempt with the Determine and Vikia tests, respectively. One participant with an invalid Vikia test had negative result on a

subsequent prick. Both the Determine test and HBsAg ELISA for this participant were negative.

Interrater reliability (study 2). Interrater agreement (κ value) was 1.00 for the Determine test and 0.99 for Vikia. There was one disagreement about the reading of a Vikia result, and this participant was finally tested negative by ELISA. This discordant result was excluded from the subsequent analysis.

Diagnostic accuracy. Diagnostic accuracy of each POC test is presented in Table 1 (for field setting, studies 1 and 2) and Table 2 (for laboratory setting, study 3). The AUROC did not vary significantly by POC: the values in the field were 0.942 (95% confidence interval [CI], 0.911 to 0.973) and 0.949 (95% CI, 0.910 to 0.987) for Determine and Vikia, respectively; in the laboratory setting, the values were 0.943 (95% CI, 0.894 to 0.992) and 0.943 (95% CI, 0.903 to 0.984) for Determine and Espline, respectively. In studies 1 and 2, the lowest serum HBsAg levels quantified by Architect that showed reactivity in the Determine and Vikia tests were 26.5 IU/ml (Table 3), while in study 3 the lowest levels that showed reactivity in the Determine and Espline tests were 2.8 IU/ml (Table 4).

Characteristics of participants with false-negative results. Of 1,000 participants examined for the POC validation, 293 were found to carry HBsAg using the reference standard tests, and there were 23 (2.3%) participants with false-negative results: five by the Determine test in study 1, one by Determine and six by both the Determine and Vikia tests in study 2, and one by Determine, four by Espline, and six by both the Determine and Espline tests in study 3 (Tables 3 and 4). Of 23 participants with false-negative results, four in studies 1 and 2 declined to have a liver assessment at the MRC clinic, leaving 8 and 11 subjects for the subsequent analysis for factors associated with false negativity in the field (studies 1 and 2) and laboratory (study 3) settings, respectively.

In the field studies, subjects with false negatives were more likely to be female ($P = 0.05$), with lower HBsAg levels ($P = 0.0002$) and lower ALT levels ($P = 0.01$) than subjects with true-positive results (Table 4). In the laboratory study, subjects with false negatives were older ($P = 0.04$) and had lower HBsAg ($P < 0.0001$) and ALT levels ($P = 0.01$) than true-positive participants (Table 4). None of subjects with false negatives were positive for HBeAg or had an ALT over the normal range (>40 IU/ml), and only one had a high viral load (>2,000 IU/ml), implying that the vast majority (94.7%, 18/19) of subjects with false-negative results were inactive carriers (26). However, POC tests missed four participants with an elevated LSM over 7.2 kPa. Three of these subjects were even in a precirrhotic range (subjects EG0203, EG0444, and EG0783). There was no HIV, HCV, or HDV coinfection in HBsAg-positive participants in studies 1 and 2 while three HBsAg-positive patients in study 3 were also positive for HIV (two with HIV-1 and one with HIV-2). They were all treatment naive and were reactive for the Determine and Espline tests. Their viral loads ranged from <50 to 18,000 IU/ml, and HBsAg levels ranged from 7 to 2,334 IU/ml.

DISCUSSION

Numerous HBsAg POC tests using immunochromatographic assays have been commercialized worldwide. However, the majority of these tests were evaluated under laboratory conditions using serum or plasma. Only a few studies assessed the performance of these tests using whole blood from venipuncture (9, 12, 16, 17, 19); none of them used capillary blood from finger prick in the field.

We studied the diagnostic accuracy of POC tests in both

TABLE 1 Diagnostic accuracy of Determine and Vikia tests in field settings (studies 1 and 2)

Test	No. of AxSYM ELISA results that were:[a]		Value for the parameter (95% CI)[b]						
	Positive	Negative	AUROC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR−
Determine from study 1	$n = 44$	$n = 252$	0.943 (0.896–0.991)	88.6 (75.4–96.2)	100.0 (98.5–100)	100.0 (91.0–100)	98.1 (95.5–99.4)	∞ (NA)	0.114 (0.05–0.26)
Positive	39	0							
Negative	5	252							
Determine from study 2	$n = 60$	$n = 417$	0.942 (0.901–0.983)	88.3 (77.4–95.2)	100.0 (99.1–100)	100.0 (93.3–100)	98.3 (93.3–100)	∞ (NA)	0.117 (0.06–0.23)
Positive	53	0							
Negative	7	417							
Determine from studies 1 and 2	$n = 104$	$n = 669$	0.942 (0.911–0.973)	88.5 (80.7–93.9)	100.0 (99.5–100)	100.0 (96.1–100)	98.2 (96.9–99.1)	∞ (NA)	0.115 (0.07–0.20)
Positive	92	0							
Negative	12	669							
Vikia from study 2	$n = 60$	$n = 416$	0.949 (0.910–0.987)	90.0 (79.5–96.2)	99.8 (98.7–100)	98.2 (90.3–100)	98.6 (96.9–99.5)	374.4 (52.8–2656)	0.100 (0.05–0.21)
Positive	54	1							
Negative	6	415							

[a] ELISA, enzyme-linked immunosorbent assay.
[b] Abbreviations: AUROC, area under the receiver operating characteristics curves; NPV, negative predictive value; PPV, positive predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NA, not applicable.

community (using whole blood from finger prick) and laboratory (using serum) settings. Our study found the following: (i) an acceptable range of diagnostic accuracy of the three tests in both the field and laboratory settings; (ii) a minimum clinical impact of the low sensitivity of these tests as false negatives were inactive carriers; (iii) excellent interrater reliability; and (iv) infrequent invalid results.

These tests are widely used and have been assessed in various studies (Table 5). In 2012, a meta-analysis reported high performance of the Determine test, with a pooled sensitivity at 98.2% (95% credible interval [CrI], 94.7 to 99.9%) and a specificity at 99.9% (95% CrI, 99.3 to 100%) (6). However, other studies cast doubt on the Determine test's high sensitivity (13, 15, 16). Sensitivity was generally acceptable, ranging from 93.6 to 100% (except in one study [10]), in HIV-negative individuals (Table 5). However, in HIV-infected individuals results were conflicting; unsatisfactory sensitivity (55.9 to 75.0%) was observed in Malawi, Ghana, and South Africa while in Tanzania and United Kingdom sensitivity was 96.0 to 100% (14, 17). Lamivudine- or tenofovir-based antiretroviral therapy may reduce HBsAg levels in coinfected people and may explain the heterogeneous sensitivity among HIV-infected individuals (17). However, a recent study assessing another HBsAg POC test in HIV-infected people in Guinea-Bissau failed to find an association between a history of lamivudine therapy and false negativity (18). In our study, most of participants were not HIV infected and none of them received HBV antiviral therapy. The sensitivities of the Determine test (88.5% [95% CI, 80.7 to 93.9%] in the field and 95.3% [95% CI, 90.5 to 98.1%] in the laboratory) were within the range reported in HIV-uninfected individuals.

To date, the Vikia test has been validated in parallel with Determine in two studies. Both studies found similar sensitivities in Vikia and Determine: 70.7% and 69.3% in Ghana and 96.5% and 93.6% in France, respectively (15, 19). Similarly, we did not find a significant difference in sensitivities between the Vikia and Determine tests in study 2 (90.0% and 88.3%, respectively). One discordant result (Vikia-positive and Determine-negative result) was observed in an HBsAg-positive participant with a very low serum HBsAg level at 14.6 IU/ml (subject EG0865).

Previously, only one study has assessed the diagnostic accuracy of the Espline test and reported sensitivity of 94.7% and specificity of 100% (11), values which were similar to our findings.

It has been reported that false-negative results of HBsAg POC tests are associated with a low HBsAg concentration, HBsAg mutants, low viral load, and certain viral genotypes (10, 15, 19, 27). Although the number of subjects with false-negative results in our study was small, subjects with false negatives tend to have inactive disease and lower HBsAg levels than subjects with true-positive results, suggesting a low clinical impact of the low sensitivity of these tests. However, of 23 subjects with false-negative results, 4 (17%) were found to have elevated LSM values by transient elastography, implying that a POC test is not a perfect tool to rule out HBV-infected individuals who require antiviral therapy due to advanced liver fibrosis in SSA.

Our study has several limitations. First, we could not obtain HBV genetic information on the HBVs in order to relate a specific mutation or viral genotype to false-negative results.

Second, we used DBS as a medium for the reference standard

TABLE 2 Diagnostic accuracy of Determine and Espline tests in laboratory settings (study 3)

Test	No. of Architect CMIAs that were:[a]		Value for the parameter (95% CI)[b]					
	Positive	Negative	AUROC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR−
Determine	$n = 148$	$n = 30$	0.943 (0.894–0.992)	95.3 (90.5–98.1)	93.3 (77.9–99.2)	98.6 (95.0–99.8)	80.0 (63.1–91.6)	14.3 (3.7–54.5)	0.051 (0.02–0.11)
Positive	141	2							
Negative	7	28							
Espline	$n = 165$	$n = 38$	0.943 (0.903–0.984)	93.9 (89.1–97.1)	94.7 (82.3–99.4)	98.7 (95.5–99.8)	78.3 (63.6–89.1)	17.8 (4.6–68.8)	0.064 (0.03–0.12)
Positive	155	2							
Negative	10	36							

[a] CMIA, chemiluminescent microparticle immunoassay.

[b] Abbreviations: AUROC, area under the receiver operating characteristics curves; NPV, negative predictive value; PPV, positive predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio.

TABLE 3 Characteristics of HBsAg-positive participants according to the results of Determine and Vikia tests in studies 1 and 2[a]

Test group or subject	Test result		Age (yr) (range)	No. and/or sex of subject(s)	HBsAg (IU/ml) (range)	HBV DNA (IU/ml) (range)	ALT (IU/ml) (range)	LSM (kPa) (range)	HBeAg status (no./total [%])
	Determine	Vikia							
Groups									
True positive ($n = 92$)			39 (30–83)	42 males[b] 50 females	875 (26.5–27,320)[c]	<50 (<50–10^8)	22 (8–191)[d]	4.7 (2.2–26.7)	Positive (1/29 [3])
False negative ($n = 12$)			42 (30–68)	2 males[b] 10 females	1.2 (0.8–25.5)[c]	334 (76–3,390)	15 (8–35)[d]	4.0 (2.5–4.9)	Positive (0/7 positive [0])
Subjects									
EG0646	Negative	Not tested	51	Female	17.59	3,390	8	4.9	Negative
EG0647	Negative	Not tested	40	Female	Missing	76	35	3.0	Missing
EG0809	Negative	Not tested	57	Male	25.50	190	15	2.5	Negative
EG0865	Negative	Positive	35	Female	14.60	339	17	4.4	Negative
EG1047	Negative	Negative	30	Female	0.80	329	21	3.8	Negative
EG1050	Negative	Negative	40	Female	1.20	699	11	4.0	Negative
EG1068	Negative	Negative	52	Female	1.00	259	12	Missing	Negative
EG1072	Negative	Negative	66	Female	1.00	824	15	4.4	Negative
SG00423	Negative	Not tested	40	Female	Declined	Declined	Declined	Declined	Declined
SG02516	Negative	Not tested	37	Male	Declined	Declined	Declined	Declined	Declined
SG05162	Negative	Negative	68	Female	Declined	Declined	Declined	Declined	Declined
SG05235	Negative	Negative	43	Female	Declined	Declined	Declined	Declined	Declined

[a] Values for the groups are medians (ranges), where indicated. Abbreviations: ALT, alanine transaminase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LSM, liver stiffness measurement.
[b] There were significantly more men in the true-positive group (42/92, or 46%) than in the false-negative group (2/12, or 17%) ($P = 0.05$).
[c] The median HBsAg level was significantly higher in the true-positive group than in the false-negative group ($P = 0.0002$).
[d] The median ALT level was significantly higher in true positive-group than in the false-negative group ($P = 0.01$).

instead of serum or plasma. The diagnostic accuracy of HBsAg detection (ELISA) using DBS compared to the accuracy with serum or plasma has been assessed in the past and found to be very good, with sensitivity and specificity at 96 to 100% and at 97 to 100%, respectively (28–30).

Currently, WHO is calling for urgent action to reduce the burden of viral hepatitis and is designing HBV guidelines for screening and treatment in low- and middle-income countries (31). This study provides important evidence to support the feasibility of a large-scale community screening program for

TABLE 4 Characteristics of HBsAg-positive participants according to the results of Determine and Espline in study 3[a]

Test group or subject	Test result		Age (yr) (range)	No. and/or sex of subject(s)	HBsAg l (IU/ml) (range)	HBV DNA (IU/ml) (range)	ALT (IU/ml) (range)	LSM (kPa) (range)	HBeAg status (no./total [%])
	Determine	Espline							
Groups									
True positive ($n = 178$)			37 (14–79)[b]	79 males[c] 99 females	7,482 (2.8–124,925)[d]	133 (<50–10^8)	24 (8–166)[e]	4.7 (2.4–15.4)	Positive (10/177 [6])
False negative ($n = 11$)			45 (32–75)[b]	4 males[c] 7 females	0.40 (0.05–17,276)[d]	192 (<50–1,286)	19 (13–26)[e]	4.7 (3.2–10.0)	Positive (0/11 [0])
Subjects									
EG0182	Negative	Negative	75	Female	0.06	<50	17	5.1	Negative
EG0203	Negative	Positive	53	Female	1.73	105	14	8.5	Negative
EG0228	Negative	Negative	42	Female	0.09	254	13	3.4	Negative
EG0245	Negative	Negative	41	Male	2.49	<50	25	7.6	Negative
EG0257	Negative	Negative	60	Female	0.42	203	18	4.0	Negative
EG0269	Negative	Negative	32	Female	0.05	192	24	3.2	Negative
EG0282	Negative	Negative	68	Female	0.23	111	20	3.6	Negative
EG0444	Positive	Negative	33	Male	10.80	235	20	10.0	Negative
EG0770	Not tested	Negative	44	Male	0.10	<50	15	4.5	Negative
EG0783	Not tested	Negative	33	Male	17276.20	215	26	8.3	Negative
EG0787	Not tested	Negative	52	Female	4.63	1,286	19	4.7	Negative

[a] Values for the groups are medians (ranges), where indicated. Abbreviations: ALT, alanine transaminase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LSM, liver stiffness measurement.
[b] The median age was significantly higher in the false-negative group than in the true positive-group ($P = 0.04$).
[c] The percentages of males in the true-positive and false-negatives groups are 44% (79/178) and 36% (4/11), respectively.
[d] The median HBsAg level was significantly higher in true-positive group than in the false-negative group ($P < 0.0001$).
[e] The median ALT level was significantly higher in the true-positive group than in the false-negative group ($P = 0.01$).

TABLE 5 Studies assessing the accuracy of the Determine, Vikia, and Espline HBsAg tests

Test and year of study	Author (reference)	Country(ies)	Manufacturer	Test setting	Population	Medium[b]	Gold standard[c]	Study design[d]	No. of true-positive results	No. of true-negative results	Sensitivity (%)	Specificity (%)
Determine												
1999	Palmer et al. (32)	Four Latin American countries	Abbott	NR[a]	NR	S	NR	CC	194	104	97.4	96.2
2000	Lien et al. (12)	Vietnam	Abbott	Local laboratory	NR	S	EIA	CC	101	99	100	100
					High-risk volunteers, pregnant women, patients with other infections (including 10 HIV^+)	S, P, venous WB with EDTA[f]	EIA	CS	16	112	100	100
2008	Randrianirina et al. (33)	Madagascar	Abbott	Local laboratory	NR	S	EIA	CC	91	200	97.8	100
2008	Nyirenda et al. (13)	Malawi	Abbott	Local laboratory[e]	Hospital patients (including 152 HIV^+)	P[e]	EIA	CS	34	160	55.9	69.4
2008	Lin et al. (10)	China	Inverness	Local laboratory	Blood donors with false-negative results (cases); negative blood donors (controls)[h]	P	EIA	CC	146	498	77.4	100
					Blood donors and hospital patients	S	EIA	CC	186	485	98.9	100
		Guinea	Inverness	Local laboratory	Blood donors	S or P	EIA	CC	180	399	94.4	100
2010	Davies et al. (14)	Malawi	Inverness	Laboratory in the UK	Treatment-naive HIV^+ patients	S	EIA	CS	25	50	100	100
2010	Geretti et al. (15)	Ghana	Inverness	Laboratory in the UK	HIV^+ patients[g]	S	CMIA, EIA	CS	140	698	69.3	100
				Local laboratory	HIV^+ patients[g]	S	CMIA	CS	178	0	N/A	100
2012	Hoffmann et al. (16)	South Africa	Abbott	Local laboratory	Treatment-naive HIV^+ patients	Capillary WB without EDTA[e]	ELISA	CS	40	933	75.0	99.6
2013	Bottero et al. (19)	France	Inverness	Local laboratory	General population	Venous WB without EDTA	ELISA	CS	47	2425	93.6	100
2013	Franzeck et al. (17)	Tanzania	Alere	Local laboratory	Treatment-naive HIV^+ patients	P, venous WB with EDTA[f]	EIA	CS	25	247	96.0	100
Vikia												
2010	Geretti et al (15)	Ghana	bioMérieux	Laboratory in the UK	HIV^+ patients[g]	S	CMIA, EIA	CS	140	698	70.7	100
2013	Bottero (19)	France	bioMérieux	Local laboratory	General population	Venous WB without EDTA	ELISA	CS	85	3843	96.5	99.9
Espline												
1997	Shibahara et al. (11)	Japan	Fujirebio	Local laboratory	Hospital patients	S	MEIA	CS	132	173	94.7	100

[a] NR, not reported.
[b] S, serum; P, plasma; WB, whole blood.
[c] EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; MEIA, microparticle enzyme immunoassay; CMIA, chemiluminescent microparticle immunoassay.
[d] CC, case-control study; CS, cross-sectional study.
[e] Confirmed with a communication with the corresponding author.
[f] Identical results were obtained by testing different type of samples.
[g] One-third of HIV-positive (HIV^{+1}) subjects received lamivudine-based therapy.
[h] The false-negative results were from a local POC (Wantai Biological Pharmacy, China).

HBV in SSA by showing acceptable diagnostic accuracy of inexpensive (<$2) HBsAg rapid POC tests using finger prick in a real community environment.

ACKNOWLEDGMENTS

We thank the Medical Research Council Laboratories in the Gambia, the local Ministry of Health and Social Welfare for supporting the project, all the study participants, and the PROLIFICA team, in particular, Ignatius Baldeh, Famara Bojang, Amie Ceesay, Mavis Foster-Nyarko, Debbo Jallow, Abdoulie Jatta, Adam Jeng, Sheriff Kolley, Yamundow Jallow Samba, Alagie Sanneh, Demba Sonko, Penda Suso, and Saydiba Tamba. We also thank Debbie Garside, the project manager of the PROLIFICA project.

This study is part of the PROLIFICA program funded by the European commission (EC FP7, P34114).

We have no conflict of interest related to this study.

REFERENCES

1. **Ott JJ, Stevens GA, Groeger J, Wiersma ST.** 2012. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine **30:**2212–2219. http://dx.doi.org/10.1016/j.vaccine.2011.12.116.
2. **Parkin DM, Sitas F, Chirenje M, Stein L, Abratt R, Wabinga H.** 2008. Part I: cancer in indigenous Africans—burden, distribution, and trends. Lancet Oncol **9:**683–692. http://dx.doi.org/10.1016/S1470-2045(08)70175-X.
3. **Jemal A, Bray F, Forman D, O'Brien M, Ferlay J, Center M, Parkin DM.** 2012. Cancer burden in Africa and opportunities for prevention. Cancer **118:**4372–4384. http://dx.doi.org/10.1002/cncr.27410.
4. **Thursz M, Cooke GS, Hall AJ.** 2010. Hepatitis B treatment in resource poor settings: time for action. Trop Med Int Health **15:**2–4. http://dx.doi.org/10.1111/j.1365-3156.2009.02410.x.
5. **Thursz M, Njie R, Lemoine M.** 2012. Hepatitis: global eradication of hepatitis B-feasible or fallacy? Nat Rev Gastroenterol Hepatol **9:**492–494. http://dx.doi.org/10.1038/nrgastro.2012.155.
6. **Shivkumar S, Peeling R, Jafari Y, Joseph L, Pai NP.** 2012. Rapid point-of-care first-line screening tests for hepatitis B infection: a meta-analysis of diagnostic accuracy (1980–2010). Am J Gastroenterol **107:**1306–1313. http://dx.doi.org/10.1038/ajg.2012.141.
7. **Hwang SH, Oh HB, Choi SE, Kim HH, Chang CL, Lee EY, Son HC.** 2008. Meta-analysis for the pooled sensitivity and specificity of hepatitis B surface antigen rapid tests. Korean J Lab Med **28:**160–168. http://dx.doi.org/10.3343/kjlm.2008.28.2.160.
8. **Mvere D, Constantine NT, Katsawde E, Tobaiwa O, Dambire S, Corcoran P.** 1996. Rapid and simple hepatitis assays: encouraging results from a blood donor population in Zimbabwe. Bull World Health Organ **74:**19–24.
9. **Akanmu AS, Esan OA, Adewuyi JO, Davies AO, Okany CC, Olatunji RO, Babalola T.** 2006. Evaluation of a rapid test kit for detection of HBsAg/eAg in whole blood: a possible method for pre-donation testing. Afr J Med Med Sci **35:**5–8.
10. **Lin YH, Wang Y, Loua A, Day GJ, Qiu Y, Nadala EC, Jr, Allain JP, Lee HH.** 2008. Evaluation of a new hepatitis B virus surface antigen rapid test with improved sensitivity. J Clin Microbiol **46:**3319–3324. http://dx.doi.org/10.1128/JCM.00498-08.
11. **Shibahara K, Sugada F, Okuse C, Yasuda K, Lino S.** 1997. Fundamental evaluation of HBs antigen and HBs antibody rapid test by immunochromatography assay. Jpn J Med Pharm Sci **38:**343–350.
12. **Lien TX, Tien NT, Chanpong GF, Cuc CT, Yen VT, Soderquist R, Laras K, Corwin A.** 2000. Evaluation of rapid diagnostic tests for the detection of human immunodeficiency virus types 1 and 2, hepatitis B surface antigen, and syphilis in Ho Chi Minh City, Vietnam. Am J Trop Med Hyg **62:**301–309.
13. **Nyirenda M, Beadsworth MB, Stephany P, Hart CA, Hart IJ, Munthali C, Beeching NJ, Zijlstra EE.** 2008. Prevalence of infection with hepatitis B and C virus and coinfection with HIV in medical inpatients in Malawi. J Infect **57:**72–77. http://dx.doi.org/10.1016/j.jinf.2008.05.004.
14. **Davies J, van Oosterhout JJ, Nyirenda M, Bowden J, Moore E, Hart IJ, Zijlstra EE, Chaponda M, Faragher B, Beeching NJ, Beadsworth MB.** 2010. Reliability of rapid testing for hepatitis B in a region of high HIV endemicity. Trans R Soc Trop Med Hyg **104:**162–164. http://dx.doi.org/10.1016/j.trstmh.2009.10.010.
15. **Geretti AM, Patel M, Sarfo FS, Chadwick D, Verheyen J, Fraune M, Garcia A, Phillips RO.** 2010. Detection of highly prevalent hepatitis B virus coinfection among HIV-seropositive persons in Ghana. J Clin Microbiol **48:**3223–3230. http://dx.doi.org/10.1128/JCM.02231-09.
16. **Hoffmann CJ, Dayal D, Cheyip M, McIntyre JA, Gray GE, Conway S, Martinson NA.** 2012. Prevalence and associations with hepatitis B and hepatitis C infection among HIV-infected adults in South Africa. Int J STD AIDS **23:**e10–e13. http://dx.doi.org/10.1258/ijsa.2009.009340.
17. **Franzeck FC, Ngwale R, Msongole B, Hamisi M, Abdul O, Henning L, Letang E, Mwaigomole G, Battegay M, Hatz C, Tanner M.** 2013. Viral hepatitis and rapid diagnostic test based screening for HBsAg in HIV-infected patients in rural Tanzania. PLoS One **8:**e58468. http://dx.doi.org/10.1371/journal.pone.0058468.
18. **Honge B, Jespersen S, Medina C, Te D, da Silva Z, Ostergaard L, Laursen A, Wejse C, Krarup H, Erikstrup C.** 2014. Hepatitis B virus surface antigen and anti-hepatitis C virus rapid tests underestimate hepatitis prevalence among HIV-infected patients. HIV Med **15:**571–576. http://dx.doi.org/10.1111/hiv.12158.
19. **Bottero J, Boyd A, Gozlan J, Lemoine M, Carrat F, Collignon A, Boo N, Dhotte P, Varsat B, Muller G, Cha O, Picard O, Nau J, Campa P, Silbermann B, Bary M, Girard PM, Lacombe K.** 2013. Performance of rapid tests for detection of HBsAg and anti-HBsAb in a large cohort, France. J Hepatol **58:**473–478. http://dx.doi.org/10.1016/j.jhep.2012.11.016.
20. **Whittle HC, Bradley AK, McLauchlan K, Ajdukiewicz AB, Howard CR, Zuckerman AJ, McGregor IA.** 1983. Hepatitis B virus infection in two Gambian villages. Lancet **i:**1203–1206.
21. **Whittle H, Inskip H, Bradley AK, McLaughlan K, Shenton F, Lamb W, Eccles J, Baker BA, Hall AJ.** 1990. The pattern of childhood hepatitis B infection in two Gambian villages. J Infect Dis **161:**1112–1115. http://dx.doi.org/10.1093/infdis/161.6.1112.
22. **Castera L, Forns X, Alberti A.** 2008. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol **48:**835–847. http://dx.doi.org/10.1016/j.jhep.2008.02.008.
23. **Lemoine M, Shimakawa Y, Njie R, Njai HF, Nayagam S, Khalil M, Goldin R, Ingiliz P, Taal M, Nyan O, Corrah T, D'Alessandro U, Thursz M.** 2014. Food intake increases liver stiffness measurements and hampers reliable values in patients with chronic hepatitis B and healthy controls: the PROLIFICA experience in the Gambia. Aliment Pharmacol Ther **39:**188–196. http://dx.doi.org/10.1111/apt.12561.
24. **Marcellin P, Ziol M, Bedossa P, Douvin C, Poupon R, de Ledinghen V, Beaugrand M.** 2009. Non-invasive assessment of liver fibrosis by stiffness measurement in patients with chronic hepatitis B. Liver Int **29:**242–247. http://dx.doi.org/10.1111/j.1478-3231.2008.01802.x.
25. **Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, Lijmer JG, Moher D, Rennie D, de Vet HC.** 2003. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med **138:**40–44. http://dx.doi.org/10.7326/0003-4819-138-1-200301070-00010.
26. **Papatheodoridis G, Buti M, Cornberg M, Janssen HL, Mutimer D, Pol S, Raimondo G, Dusheiko G, Lok A, Marcellin P, European Association for the Study of the Liver.** 2012. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol **57:**167–185. http://dx.doi.org/10.1016/j.jhep.2012.02.010.
27. **Scheiblauer H, El-Nageh M, Diaz S, Nick S, Zeichhardt H, Grunert HP, Prince A.** 2010. Performance evaluation of 70 hepatitis B virus (HBV) surface antigen (HBsAg) assays from around the world by a geographically diverse panel with an array of HBV genotypes and HBsAg subtypes. Vox Sang **98:**403–414. http://dx.doi.org/10.1111/j.1423-0410.2009.01272.x.
28. **Farghaly AM, Mohsen A, Omar ME, Khalil MA, Gaber MA.** 1990. Synthesis of 3-aryl-2-substituted-4(3H)-quinazolines as potential antimicrobial agents. Farmaco **45:**431–438.
29. **Villar LM, de Oliveira JC, Cruz HM, Yoshida CF, Lampe E, Lewis-Ximenez LL.** 2011. Assessment of dried blood spot samples as a simple method for detection of hepatitis B virus markers. J Med Virol **83:**1522–1529. http://dx.doi.org/10.1002/jmv.22138.
30. **Kania D, Bekale AM, Nagot N, Mondain AM, Ottomani L, Meda N, Traore M, Ouedraogo JB, Ducos J, Van de Perre P, Tuaillon E.** 2013. Combining rapid diagnostic tests and dried blood spot assays for point-of-care testing of human immunodeficiency virus, hepatitis B and hepatitis C infections in Burkina Faso, West Africa. Clin Microbiol Infect **19:**E533–E541. http://dx.doi.org/10.1111/1469-0691.12292.

31. **World Health Organization.** 2014. Call to action to scale up global hepatitis response. World Health Organization, Geneva, Switzerland. http://www.who.int/hiv/mediacentre/news/hep_calltoaction2014/en/.
32. **Palmer CJ, Cuadrado RR, Koenig E, Dubon JM, Dowe G, Palmer DA.** 1999. Multicenter evaluation of the Determine™ rapid tests for the diagnosis of HIV, hepatitis B surface antigen, and syphilis, abstr 1582. Abstr 39th Intersci Conf Antimicrob Agents Chemother, 26 to 29 September 1999. American Society for Microbiology, Washington, DC.
33. **Randrianirina F, Carod JF, Ratsima E, Chretien JB, Richard V, Talarmin A.** 2008. Evaluation of the performance of four rapid tests for detection of hepatitis B surface antigen in Antananarivo, Madagascar. J Virol Methods **151:** 294–297. http://dx.doi.org/10.1016/j.jviromet.2008.03.019.